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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/247,054	02/09/99	ANTONIOU M	CACO-0045

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EXAMINER	
BAKER, A	
ART UNIT	**PAPER NUMBER**
1632	5

DATE MAILED: 07/09/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trad mark

Office Action Summary

Application No.	Applicant(s)
09/247,054	Antoniou et al.
Examiner	**Group Art Unit**
Anne-Marie Baker, Ph.D.	1632

☐ Responsive to communication(s) filed on _____ .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 1-25 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 1-25 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☒ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 4

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☒ Notice to Comply with Sequence Requirements.

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Claims 1-25 are pending in the instant application.

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Applicant is given the same shortened statutory period set forth for response to the instant office action within which to comply with the sequence rules, 37 CFR 1.821 - 1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a). In no case may an applicant extend the period for reply beyond the SIX MONTH statutory period. Direct the reply to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the reply.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claim 24 is drawn to a transgenic animal which would encompass human beings which are non-statutory. Inclusion of the phrase "non-human" would be remedial.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 22 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is drawn to a method for expressing a gene of interest in cells of a specific tissue-type, wherein a self-replicating episomal DNA expression vector or a pair of vectors is administered to a mammal.

The specification is not enabling for *in vivo* use of the gene expression vectors because gene transfer and expression from gene delivery vectors is highly unpredictable. Although *in vitro* expression from several exemplary vectors is described in the specification, no examples of *in vivo* delivery of the vectors is described. At the time the application was filed, the art of administering any type of gene expression vector to an individual so as to provide a tangible therapeutic benefit was poorly developed and unpredictable. The NIH ad hoc committee to assess the current status and promise of gene therapy reported in December 1995 that "clinical efficacy has not been definitively demonstrated at this time in any gene therapy protocol, despite anecdotal claims...," and that "significant problems remain in all basic aspects of gene therapy" (Orkin and Motulsky, p. 1). In a review article published in Scientific American in June 1997, Theodore Friedmann discusses the technical barriers which have so far prevented successful gene therapy, and states "So far, however, no approach has definitively improved the health of a single one of the more than 2,000 patients who have enrolled in gene therapy trials worldwide" (p. 96). In a review article published in Nature in

September 1997, Verma et al. states "Although more than 200 clinical trials are currently underway worldwide, with hundreds of patients enrolled, there is still no single outcome that we can point to as a success story" (p. 239). Although there are many examples of therapeutically useful nucleic acid sequences given on pp. 23-24 of the specification, any of which can be incorporated into an episomal expression vector, the specification fails to provide an enabling disclosure as to how one would achieve any therapeutic benefit using vectors of the type disclosed in the instant invention. Furthermore, the episomes of the claimed invention would not be expected to be stably inherited because they do not have a centromere. In view of the recognized obstacles to successful use of gene expression vectors *in vivo*, the high degree of unpredictability in the art of gene therapy, and the lack of specific exemplification in the disclosure, one skilled in the art would not have considered the *in vitro* examples provided in the specification to be correlative with the successful *in vivo* use of the claimed invention. Given the lack of guidance or working examples in the specification showing how to use the claimed invention successfully in a living animal or human, undue experimentation would have been required by one skilled in the art at the time the application was filed to practice the claimed invention.

In regard to Claim 24, drawn to the transgenic animal containing cells which contain the episomal expression vector of the instant invention, the specification fails to provide any guidance in the use of the vectors to obtain *in vivo* expression in a transgenic animal. Several transgenic animal models are contemplated in the specification (p.38) such as those wherein the transgenic animal has a tumor and the episomal expression vector is designed so that expression of the selected gene is strictly limited to the tumor cells. However, the specification does not describe how to achieve tumor-restricted gene expression using the episomal vectors of the instant invention. No guidance is given as to how one would have prepared such vectors, which LCRs could be used to restrict expression to which types of tumors, or how one would use

such an animal model once developed. Long-term production of a protein of interest in a transgenic animal is also contemplated, but no guidance is provided in the specification as to how to achieve long-term expression in any particular tissue-type and no examples are given. Thus, undue experimentation would have been required by one skilled in the art to achieve expression of a transgene in a transgenic animal using the vectors described.

The specification fails to provide an enabling disclosure for the preparation of any and all animals harboring an episomal expression vector of the type claimed because the only example contemplated in the specification is that of a transgenic animal expressing the human globin gene in red cells using the β-globin LCR or the HS3 and HS4 combination or HS3 alone, or a transgenic animal expressing a protein of interest in its milk using the macrophage/lysozyme LCR. However, no guidance is given regarding how to use the transgenic animals once obtained. Furthermore, due to the unpredictability in the art, as discussed in more detail below, the phenotype of the contemplated transgenic animal is not known.

The specification fails to provide an enabling disclosure for the preparation of any species of transgenic animal of the type claimed because the phenotype of a transgenic animal cannot be predicted. In the absence of a transgene-dependent phenotype, one skilled in the art would not know how to use the claimed animals. The phenotype of any species of animal having cells which contain the expression vector of Claim 1 or the pair of vectors of Claim 12 as recited in the claim, cannot be predicted. The specification does not teach what phenotype would be expected in any species of transgenic animal of the type claimed. Furthermore, the specification does not adequately teach how one would have prepared any and all transgenic animals expressing a gene of interest from a self-replicating episomal expression vector of the type recited in the claims. The mere capability to perform gene transfer in any given species is not enabling for the claimed transgenic animals because a predictable phenotype cannot be achieved by simply introducing a transgene

encoding a gene of interest. While gene transfer techniques are well-developed for a number of species, especially in the mouse, methods for achieving the desired level of transgene expression in appropriate tissues are less well-established. The introduction of DNA into the mammalian genome can ordinarily be achieved most reliably by microinjection or retrovirus-mediated gene transfer. However, the state of the art for transgenics is unpredictable because the method of gene transfer typically relies on random integration of the transgene construct. Insertional inactivation of endogenous genes and position effects (see Wall, 1996, p. 61, paragraph 3) can dramatically influence the phenotype of the resultant transgenic animal. Integration of the transgene near highly active genes or, alternatively, in a transcriptionally inactive region, can influence its level of expression. Furthermore, expression of the transgene and the effect of transgene expression on the phenotype of the transgenic animal depends on the particular gene construct used, to an unpredictable extent. The particular genetic elements required for appropriate expression varies from species to species. Thus, a construct that confers the desired phenotype in a mouse will not necessarily achieve the same result in a rat. Wall (1996) reports that our lack of understanding of essential genetic control elements makes it difficult to design transgenes with predictable behavior (p. 61, paragraph 3). This is especially relevant for species in which genetic studies are less advanced than in the mouse. Thus, the species-specific requirements for transgene design introduces an additional level of unpredictability associated with the development of transgenic animals. Furthermore, there are inherent physiological differences between mice, birds, cows, fish, pigs, etc. that can affect the phenotype in an unpredictable manner. In the absence of representative working examples, the existence of any phenotypic alteration resulting from the introduction of an episomal expression vector of the type claimed in any species of animal, is highly unpredictable. Without knowing the phenotype of the transgenic mouse, fish, cow, pig, or bird, one of skill in the art would not know how to use the animal. Given the lack of working examples, the limited guidance in the specification, and the

unpredictability in the art, one of ordinary skill in the art would have been required to engage in undue experimentation in order to make and use the claimed transgenic animals.

While the species-specific requirements for transgene design are not clearly understood, examples in the literature demonstrate that even closely related species carrying the same transgene construct can exhibit widely varying phenotypes. For example, several animal models of human diseases have relied on transgenic rats when the development of mouse models was not feasible. Mullins et al., 1990 produced outbred Sprague-Dawley x WKY rats with hypertension caused by expression of a mouse *Ren-2* renin transgene. Hammer et al., 1990 describe spontaneous inflammatory disease in inbred Fischer and Lewis rats expressing human class I major histocompatibility allele HLA-B27 and human β_2-microglobulin transgenes. Both investigations were preceded by the failure to develop human disease-like symptoms in transgenic mice (Mullins et al., 1989; Taurog et al., 1988) expressing the same transgenes that successfully caused the desired symptoms in transgenic rats.

Given that specific phenotypic alterations cannot be predictably achieved by merely transferring a gene of interest into an animal, specific guidance must be provided to enable the instant invention. The specification must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The claims cover any species of transgenic animal having any gene of interest linked to any promoter in combination with an LCR or component thereof, but the specification does not enable the full scope of the claimed compositions. In the absence of disclosure of transgenic animals, exhibiting a transgene-dependent phenotype, representative of the full scope of the claimed transgenic animals, undue experimentation would have been required to make and use the claimed animals.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-21 are indefinite in regard to the intended use of the self-replicating episomal DNA expression vector for expressing a gene of interest in a host cell in a tissue-restricted manner because it is unclear whether the host cell is expressing the gene *in vivo* or *in vitro*. If *in vitro* use of the vector is intended then it is unclear how the expression could be said to be tissue-restricted, since tissue-specific expression must be analyzed in the context of the whole animal.

Claims 1-21 are indefinite in the recitation of the phrase "gene of interest" because a gene of interest can be virtually any gene. Therefore it is unclear what is encompassed by the claim. The metes and bounds of the claims are not clearly set forth.

Claims 5, 14, and 16 are indefinite in the recitation of "the β-globin LCR consisting essentially of" because it is unclear what specifically it is that is essential.

Claims 12-21 are indefinite in the recitation of "the replication protein being necessary for replication of said origin of replication" because it is unclear whether the origin of replication is that of (ia) or (iia) and because it is unclear whether the origin of replication of (ia) is the same as the origin of replication of (iia).

Claim 25 is indefinite in its recitation of "the LCR or component thereof which is only active in the cell line in which the LCR when integrated is active" because the meaning of the phrase cannot be deciphered.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a

whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-3, 5-14, and 16-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yates et al. (1985), Sadelain et al. (1995), Greaves et al. (1989), Grosveld et al. (1987), Ustav et al. (1991), and Svensson et al. (1996).

The instant invention is drawn to a self-replicating episomal DNA expression vector for expressing a gene of interest in a host cell in a tissue-restricted manner wherein the vector comprises a self-replicating origin of replication and a locus control region.

Yates et al. (1985) describe plasmids derived from Epstein-Barr virus that replicate stably in mammalian cells and are maintained as plasmids in most transformed cells. The origin of replication *oriP* was identified as the genetic element necessary for the plasmid to self-replicate and the *trans*-acting gene EBNA-1 was shown to be necessary for *oriP* function. Circular DNAs containing *oriP*, the EBNA-1 gene, and a selectable marker were found to replicate autonomously in cultured cells. Yates et al. do not teach the use of a locus control region in the EBV-based vector to confer tissue-specific expression.

Sadelain et al. (1995) teach a retroviral vector bearing the human β-globin gene and the LCR core sites HS2, HS3, and HS4. The vector confers erythroid-specific expression of the β-globin gene. Expression in transduced murine erythroleukemia (MEL) cells indicated some variation between different clones (p. 6728 column 2, paragraph 2). Sadelain et al. do not teach the use of the locus control region in self-replicating vectors.

Greaves et al. (1989) describe the locus control region of the human CD2 gene. DNAase I hypersensitive sites in the 3'-flanking region of the human CD2 gene conferred copy-number dependent, T-cell specific expression of a linked human CD2 minigene, independent of the position of integration in the genome of a transgenic mouse. A 28.5 kb genomic fragment of the CD2 gene was shown to be sufficient for

high-level, tissue-specific expression (p. 979 column 2, paragraph 3). The lack of position effects indicated that the LCR-containing fragment contained sequences similar to those associated with the DNAase I hypersensitive sites in the β-globin gene (p.979 column 2, paragraph 2). Greaves et al. do not teach the use of the locus control region in self-replicating vectors.

Grosveld et al. (1987) teach that the 5' and 3' flanking regions of the human β-globin locus are characterized by erythroid-specific DNAase I-superhypersensitive sites and that the presence of these regions confers tissue-specific, position-independent expression in transgenic mice. As currently defined, these regions encompass the locus control region of the β-globin gene. Grosveld et al. do not teach the use of the β-globin LCR in self-replicating vectors.

Ustav et al. (1991) teach that bovine papillomavirus is maintained as an episome with a constant copy number in transformed cells and is stably inherited. Furthermore, they showed that the transactivators E1 and E2 are required for replication (p. 453, column 2, paragraph 2). E1 and E2 are both necessary and sufficient for replication of BPV in transformed cells.

Svensson et al. (1996) teach that replication-defective viruses, such as replication-defective adenoviruses (RdAd), are useful for gene delivery because they are relatively safe, due to their inability to replicate *in vivo*, and can still be generated in very high concentrations by replicating them in cells that express the gene products essential for replication (e.g. the E1 gene products essential for replication of adenoviruses). Following infection, the adenovirus is maintained as a linear episome. Thus the reference teaches the concept of using an episomal vector carrying a transgene while the replication factors are encoded on a separate piece of DNA. In this case, the genomic DNA of the cell.

Since it would have been desirable to achieve tissue-specific expression using episomal vectors for gene transfer one would have been motivated to incorporate LCR sequences into episomes of various types in

order to make gene transfer vectors that replicate independently of the host cell chromosomes. One would have had a reasonable expectation of success because LCRs have been successfully employed to confer tissue-specific expression of exogenous genes integrated into the host cell chromosome (see e.g. Sadelain et al., p. 6728, column 2, paragraph 2). Furthermore, it would have been desirable to put the replication factors on a separate episome so that the episome encoding the replication factors could optionally be included in or excluded from the transfected cell, thereby allowing the first vector encoding the transgene to be used in a replication-defective manner, if necessary. Also, larger DNA sequences could thereby be inserted into the episomal vector carrying the gene of interest, because the sequences encoding the replication factors (e.g., E1 and E2 of papilloma virus) would be supplied by a separate episomal vector. Therefore, it would have been obvious to one of skill in the art at the time of the invention to have included known LCR sequences in episomal vectors in order to have achieved tissue-specific expression and to have placed the gene of interest and the replication factors on separate episomes that could have optionally been co-transfected into cultured cells.

One would have been motivated to have combined the teachings of Yates et al., Sadelain et al., Greaves et al., Grosveld et al., Ustav et al., and Svensson et al. in order to have generated self-replicating, tissue-specific episomal expression vectors.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yates et al. (1985), Sadelain et al (1995)., Greaves et al. (1989), Grosveld et al. (1987), Ustav et al (1991)., and Svensson et al. (1996) as applied to claims 1-3, 5-14, and 16-21 above, and further in view of Chapman et al. (1991).

Chapman et al. (1991) disclose the transfection of cultured mammalian cells with several expression vectors encoding a variety of proteins. It is common practice to transfect cultured host cells for the *in vitro* expression of a protein of interest.

As discussed above, it would have been obvious to include known LCR sequences in episomal vectors in order to achieve tissue-specific expression and to have placed the gene of interest and the replication factors on separate episomes that could have optionally been co-transfected into cultured cells. These vectors could have then been used to obtain tissue-specific expression of a gene of interest in a host cell in culture. As disclosed by Chapman et al., it is common practice to express a protein of interest in cultured host cells. One would have anticipated a reasonable expectation of success because the function of the genetic elements required to construct the claimed vectors are well-known in the art, and only standard molecular biology techniques are required to construct the claimed vectors, and only standard culturing techniques are required to express a gene of interest in a host cell in culture. Therefore, it would have been obvious to one of skill in the art at the time of the invention to have made recombinant constructs of the type claimed and to have used them to express a gene of interest in a host cell in culture.

One would have been motivated to have combined the teachings of Yates et al., Sadelain et al., Greaves et al., Grosveld et al., Ustav et al., Svensson et al., and Chapman et al. in order to have generated self-replicating, tissue-specific episomal expression vectors and to use the vectors to express a gene of interest in a host cell in culture.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yates et al. (1985), Sadelain et al (1995)., Greaves et al. (1989), Grosveld et al. (1987), Ustav et al (1991)., and Svensson et al. (1996) as applied to claims 1-3, 5-14, and 16-21 above, and further in view of Chapman et al. (1991).

Chapman et al. (1991) disclose the effect of intron A from human cytomegalovirus immediate early gene on heterologous expression in mammalian cells.

Since it is common practice to assess the function of genetic regulatory elements in transfected cell lines, wherein the regulatory element is operably linked to a marker gene, as disclosed by Chapman et al., one skilled in the art would have been motivated to construct an episomal expression vector of the type claimed using candidate LCR sequences to assess the capability of the genetic element to direct tissue-restricted expression of a linked gene. One would have anticipated a reasonable expectation of success because assessing the function of genetic regulatory elements in cultured cell lines is routine experimentation and only standard molecular biology techniques and standard culture techniques are required to perform the requisite assays. Therefore, it would have been obvious to one of skill in the art at the time of the invention to have made recombinant constructs of the type claimed using candidate LCR sequences linked to a marker gene to look assess the capacity of the LCR sequence to direct tissue-restricted expression of the marker gene.

One would have been motivated to have combined the teachings of Yates et al., Sadelain et al., Greaves et al., Grosveld et al., Ustav et al., Svensson et al., and Chapman et al. in order to develop test constructs that could be assayed for function in cultured host cells.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Upon overcoming the 35 U.S.C. 112, second paragraph rejections, Claims 4 and 15 would be allowable if written in independent form.

No claim is allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne-Marie Baker whose telephone number is (703) 306-9155. The examiner can normally be reached Monday through Friday from 8:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Stanton, can be reached on (703) 308-2801. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Anne-Marie Baker, Ph.D.
July 5, 1999

Deborah Crouch

DEBORAH CROUCH
PRIMARY EXAMINER
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